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RESPONSES OF CAROTID BODY CHEMOSENSORY ACTIVITY AND BLOOD FLOW TO STIMULATION OF SYMPATHETIC NERVES IN THE CAT

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SUMMARY

1. The effects of electrical stimulation of sympathetic nerves on sinus nerve chemosensory activity and carotid body blood flow were investigated in anaesthetized cats.

2. Two categories, designated as types I and II, of excitatory responses of chemosensory discharges to sympathetic stimulation were distinguished. Type I responses displayed elevations in impulse frequencies which were usually maximal in the initial 10–20 sec of stimulation, resisted α-adrenoceptor antagonism induced by phentolamine or phenoxybenzamine and were enhanced after administration of the dopamine antagonist, haloperidol. Type II responses showed increases in impulse frequencies which became more pronounced as stimulation progressed. These responses were susceptible to α-adrenoceptor blockade, were unaffected by haloperidol administration and were usually recorded during systemic hypotension.

3. Inhibitory changes due to activation of sympathetic fibres were recorded in 10 % of chemosensory preparations. These effects were usually either abolished or replaced by type I excitatory responses after haloperidol administration.

4. Sympathetic stimulation caused reductions of carotid body blood flow during both natural and artificial perfusion of the organ. This effect was abolished or considerably attenuated by α-adrenoceptor antagonism and was unaffected by haloperidol administration.

5. Possible mechanisms which could account for the influences of sympathetic stimulation on chemoreceptor activity and carotid body blood flow are discussed. It is concluded that inhibitory and type I excitatory responses probably arise from activation of sympathetic fibres with non-vascular terminations within the carotid body. Type II excitatory responses are most likely due to blood flow changes.

INTRODUCTION

The increase in the frequency of sinus nerve chemosensory discharges during stimulation of the sympathetic nerve supply to the carotid body (glomus caroticum) (Floyd & Neil, 1952; Eyzaguirre & Lewin, 1961) has been generally explained by stagnant asphyxial excitation of chemoreceptive elements, the consequence of vasoconstriction (Daly, Lambertsen & Schweitzer, 1954; Purves, 1970*b*). Evidence

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in favour of this explanation is that sympathetic stimulation is without effect on chemosensory discharges in circumstances in which flow changes do not operate, as in the superfused carotid body preparation (Eyzaguirre & Lewin, 1961; Belmonte & Eyzaguirre, 1974) or during total abolition of carotid body blood flow (McCloskey, 1975). However, in a variety of animals, histological studies have shown noradrenergic nerve endings in close proximity to not only carotid body blood vessels but also glomeral cells and even nerve terminals (see Verna, 1979, and Discussion). A nerve supply to the type I cells in the rat, consisting of cervical preganglionic sympathetic fibres, has also been described (McDonald & Mitchell, 1975).

These studies indicate the possibility that sympathetic fibres can modulate chemoreceptor activity by non-vascular mechanisms and the present investigation was undertaken to explore whether such a mechanism exists. Sympathetic influences on chemosensory discharges recorded from the sinus nerve and on carotid body venous outflow were studied. Preliminary findings of a part of this investigation were presented in a Communication to the Physiological Society (O'Regan, 1976*b*).

METHODS

General procedures. Fifty-five cats of either sex (1·2–4·8 kg in weight) were anaesthetized by pentobarbitone sodium (Sagatal, May & Baker, 42–48 mg/kg I.P.) Cannulae were inserted into a femoral artery, a femoral vein and the trachea low in the neck. Pentobarbitone (6–12 mg) and heparin (Leo Laboratories, 1000 i.u./kg) were periodically injected I.V. Systemic arterial blood pressure (B.P.) was measured using a pressure transducer and electromanometer (Siemens, EMT 34: EMT 311), was displayed on an oscilloscope (Tektronix, 502A) and was recorded on a channel of a direct-writing ink-jet recorder (Siemens, Mingograf 34). Arterial blood samples were periodically withdrawn for determination of P_{O_2}, P_{CO_2} and pH. (Radiometer, PHA 927b; pH meter 27, AMI I). Non-respiratory acidosis was corrected by I.V. administration of sodium bicarbonate (1–5 mM/kg). Rectal temperature was continuously monitored and maintained at 37 °C using an electric blanket (Palmer, Homeothermic Blanket System).

The parts of the trachea, larynx, pharynx and oesophagus lying between the hyoid bone and the insertion of the tracheal cannula were either removed or reflected cranially to expose the carotid bifurcations. It was usual to use the left side of the neck for experimental purposes. A polyethylene cannula, introduced into either a lingual or dorsal muscular artery, was used to inject drugs into the carotid artery (I.C.) and to measure the pressure (Siemens, transducer, 746; electromanometer, EMT 311) supplying the carotid body during artificial perfusion of the organ (see later). Perfusion pressure levels were monitored on an oscilloscope (Tektronix, 503) and recorded on the ink-jet recorder.

Artificial ventilation with air was carried out in twenty-three cats either because of disorders of pulmonary gas exchange or because cervical muscular movements interfered with chemosensory recordings. Spontaneous respiration was abolished by I.V. injections of gallamine triethiodide (Flaxedil, May & Baker, 4–8 mg/kg). Thereafter, gallamine (4 mg/kg) was administered I.V. together with pentobarbitone at hourly intervals. The stroke volume of a Starling pump, operating at a frequency of 31 cycles/min, was varied between 25 and 50 ml. in order to maintain satisfactory levels of arterial P_{O_2} (90–110 mmHg), P_{CO_2} (25–35 mmHg) and pH (7·38–7·45).

Measurements of carotid body blood flow. The technique used to collect and measure the venous outflow from the carotid body has been described in detail elsewhere (Neil & O'Regan, 1971).

Recording of chemosensory activity. Afferent activity was recorded from filaments peeled off the cut sinus nerve using saline-wick electrodes. Impulse activity was led to an amplifier (Grass, P16) and from thence to an audiomonitor and an oscilloscope (Tektronix, 502A) for preliminary examination. Filaments were further dissected until they contained a single or a few active chemosensory units. Afferent activity, initially recognized by its random pattern of discharge, was proved to be of chemoreceptor origin by observing appropriate responses during alterations of the oxygen content of the inspired air. Additionally, sodium cyanide (5–20 μg I.C.) markedly increased

the discharge rates of chemosensory units. The output of the amplifier was led to the ink-jet recorder and to a spike processor (Digitimer, D130) which was used to count the number of chemosensory impulses. The spike processor also permitted, with the aid of an oscilloscope, pulse height discriminator levels to be adjusted in order to eliminate stimulus artifacts from the chemosensory recordings during electrical stimulation of sympathetic nerves. The output of the processor was recorded on the ink-jet recorder. Photographic records were obtained from a motorized camera (Nihon Kohden) attached to an oscilloscope (Tektronix, 502A). During sympathetic stimulation some afferent filaments containing active chemosensory units also displayed potentials arising from activation of post-ganglionic fibres coursing centrally in the sinus nerve (Eyzaguirre & Uchizono, 1961). These filaments were further dissected to remove the sympathetic fibres. If this was unsuccessful the filaments were discarded and new chemosensory preparations obtained.

Artificial perfusion of the carotid body. Artificial perfusion of the carotid body was carried out in thirteen cats using a technique similar to that described by Purves (1970*a*). All arteries arising from the common and external carotid arteries in the neck, apart from those supplying the carotid body and superior cervical ganglion, were cut between ligatures. Polyethylene tubing in the form of a loop, which contained a side arm connected to a three-way tap, was inserted into the common carotid artery low in the neck. Blood contained in the side arm, loop and prepared segment of common and external carotid arteries could be put in contact with a bottle containing air under pressure by suitable adjustment of the three-way tap. Before artificial perfusion the side arm was filled with carotid arterial blood. Clamps were then applied to the common carotid artery caudad to the loop and the external carotid artery cephalad to the lingual artery. Pressure applied to the blood contained in the prepared segment of artery was continuously monitored using a lingual artery cannula. Perfusion pressure was adjusted until desired values of carotid body blood flow and chemosensory impulse frequency were obtained, and provided stable values were present, the carotid body was then perfused for a further period of time varying from 3 to 6 min. During artificial perfusion the carotid body was supplied with blood which remained in the arterial segment after applying the arterial clamps. Slight reductions of perfusion pressure, occurring as blood drained from the arterial segment, were re-adjusted during perfusion. In the period between episodes of artificial perfusion the carotid body was supplied with carotid arterial blood at the prevailing level of B.P.

Stimulation of sympathetic nerves. Preganglionic cervical sympathetic trunks were cut low in the neck and freed from the vagi and aortic nerves. Ganglioglomerular nerves, the major source of post-ganglionic sympathetic fibres supplying the carotid body (Eyzaguirre & Uchizono, 1961), were cut close to their origin from the superior cervical ganglia. The distal ends of these nerves were electrically stimulated (5–15 V, 10–20 Hz, 0·5–1 msec) for periods of between 15 and 120 sec using an electronic stimulator (Grass, S88), a stimulus isolation unit (Grass, SIU 5) and bipolar platinum electrodes. Stimuli were adequate during preganglionic sympathetic stimulation to cause maximal dilatation of the ipsilateral pupil. Electrodes were either covered by a pledget of cotton-wool soaked in warm mineral oil or immersed in a pool of the same liquid. Occasionally, B.P. fell during preganglionic sympathetic stimulation. These changes were eliminated when the aortic nerve was cut at its junction with the superior laryngeal nerve.

Analysis of results. Impulse frequencies of chemosensory discharges were counted before, during and after bouts of electrical stimulation of sympathetic nerves. Because chemoreceptor activity has an irregular pattern of discharge, it was necessary to distinguish excitatory or inhibitory effects during sympathetic stimulation from changes which could have arisen from random fluctuations of impulse frequency.

The statistical significance of differences between the discharge rates in individual chemosensory preparations was assessed on the assumption of a Poisson distribution. The mean rates of discharge during and after stimulation were compared with the mean rates before using a normal approximation

$$Z = \frac{x_1 - x_2}{\sqrt{x_1/n_1 + x_2/n_2}}.$$

where $x_1 x_2$ are the mean rates per second and $n_1 n_2$ are the time intervals in seconds over which the average rates were calculated (Armitage, 1971). In a number of chemosensory preparations changes in discharge rates were not present throughout the entire period of stimulation; in these circumstances comparison was made between control rates and rates observed during defined intervals of 15–20 sec during the stimulation period. Differences in rates were considered to be significant with $P < 0{\cdot}05$.

Values of mean systemic arterial pressure were obtained from recordings of pulsatile pressure by adding one third of the pulse pressure to the diastolic value.

Administration of drugs. Drugs used to investigate the mechanisms of sympathetic effects were: acetylcholine chloride, atropine sulphate, dopamine hydrochloride, DL-propranolol hydrochloride, hexamethonium bromide, haloperidol hydrochoride (Janssen), 2% lignocaine hydrochloride (Astra), mecamylamine hydrochloride, noradrenaline bitartrate, phenoxybenzamine hydrochloride (Smith, Kline & French), phentolamine mesylate (Ciba). Except where stated, drugs were obtained from Sigma Chemical Co. and the doses used are expressed as the weight of the salts. On the day of the experiment catecholamines and acetylcholine were prepared in modified Ringer–Locke solution containing (mM) NaCl 153, KCl 5·5, $CaCl_2$ 2·2, $NaHCO_3$ 2·0 (pH adjusted to 7·4 at 37 °C). Catecholamines were, as far as possible, protected from exposure to light and the solution in which they were dissolved also contained ascorbic acid (0·2 mg/ml.). Other drugs, apart from phenoxybenzamine, were dissolved in a 154 mM-NaCl solution (pH 7·4) and used within a week of preparation. Phenoxybenzamine was dissolved in propylene glycol and only injected I.V.

The cannulae (volumes 0·1–0·15 ml.) inserted into the carotid artery for I.C. injections were first filled with the solution containing the drug to be injected and a rapid injection of between 0·1 and 0·5 ml. was then carried out. In the interval between injections the cannulae were filled with saline solutions.

RESULTS

1. *The effects of electrical stimulation of the distal end of the cut preganglionic cervical sympathetic trunk on chemosensory discharges*

Table 1 summarizes the results obtained in this investigation and some earlier studies. The effects of sympathetic stimulation are those which were observed within 2–5 hr of inducing anaesthesia and before administration of drugs known to affect transmission in the autonomic nervous system. Eleven preparations whose responses to preganglionic sympathetic stimulation were tested in association with measurements of carotid body blood flow are included in the data (see later). Overall, 188 chemosensory preparations were examined. A statistical analysis showed that stimulation of the preganglionic trunk enhanced the discharges of seventy-three, caused depressive changes in eighteen and had no or insigificant effects in ninety-seven.

Excitatory responses

Two categories of excitatory responses to preganglionic sympathetic stimulation were distinguished based on the temporal pattern of responses and the influences of catecholaminoceptor antagonists upon these responses. For convenience, these categories have been designated as type I and type II.

Type I excitatory responses. The features which distinguished type I responses were (*a*) accentuations of chemosensory impulse frequency which were usually maximal in the initial 10–20 sec of sympathetic stimulation, (*b*) exaggeration or development of the excitatory effects after administration of the dopamine antagonist, haloperidol, and (*c*) resistance to α-adrenoceptor antagonism. Type I responses accounted for 66% of all excitatory effects observed during stimulation of the preganglionic trunk.

An example of the usual pattern of type I responses (73% of preparations) can be seen in Fig. 1. Chemosensory discharge rates were markedly increased relative to control values throughout stimulation, with maximal changes occurring in the initial stages. Discharge rates gradually recovered to levels not significantly different from control within 30 sec of the end of stimulation. In 19% of preparations displaying type I responses excitatory changes were limited to the initial 5–20 sec of stimulation.

TABLE 1. Effects of sympathetic stimulation on chemosensory discharges recorded from the sinus nerve

Series (reference)	Nerve trunk stimulated*	No. of experiments	No. of preparations	Effects		
				None	Excitation	Inhibition
I (O'Regan, 1976*a*)	PGN	9	34	22	10	2
	GGN	5	10	5	5	0
II (Murphy & O'Regan, 1977)	PGN	14	28	13	12	3
III (present study)	PGN	33	126	62	51	13
	GGN	11	29	13	13	3
			Summary			
I, II and III	PGN	56	188	97	73	18
	GGN	16	39	18	18	3

* PGN, preganglionic trunk; GGN, ganglioglomerular nerve.

Elevations of discharge frequencies remaining at much the same level throughout stimulation were recorded in 8% of preparations. Post-stimulatory excitation of discharges lasting 5–30 sec was usually associated with type I responses (66% of preparations).

Haloperidol, in doses (1–3 mg/kg I.V.; 0·2–1 mg I.C.) adequate in themselves to abolish or considerably reduce the inhibitory effects of dopamine (1–5 μg I.C.) on chemosensory discharges, caused enhancement of any type I responses already present and led to the appearance of this effect in chemosensory preparations whose impulse frequencies had previously been either unaffected or depressed during preganglionic sympathetic stimulation (Figs. 2, 3 and 7). As shown in Fig. 2*B*,

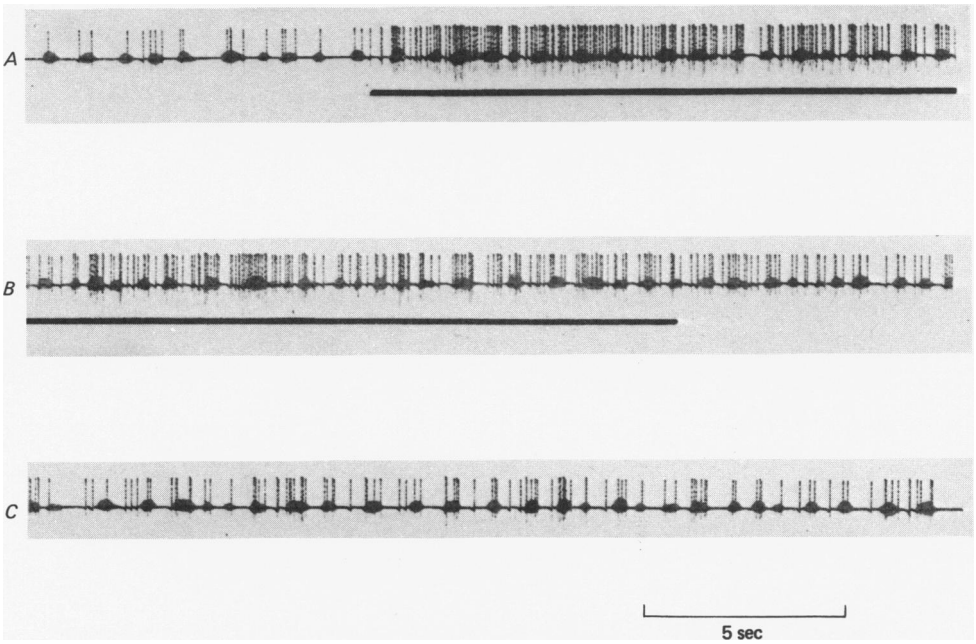

Fig. 1. Chemosensory activity recorded from a filament of the cut sinus nerve. Period of electrical stimulation (9 V, 20 Hz, 0·6 msec) of the preganglionic sympathetic trunk is indicated by the bar underneath recording. *A* to *C*, continuous recording. In this and the subsequent Figure a spike processor was used to eliminate stimulus artifacts from the chemosensory recordings.

sympathetic stimulation caused a more significant increase in chemosensory impulse frequency after an I.C. injection of haloperidol than was recorded before administration of the drug (Fig. 2*A*). Chemosensory discharge rates increased, sometimes markedly, after haloperidol administration, an effect which was usually associated with reductions of between 10 and 50 mmHg in the mean levels of B.P. Sympathetic stimulation was still effective in exciting chemosensory discharges despite widely differing prestimulatory levels of impulse frequencies (Fig. 2*C*). Type I responses were no longer elicited (Fig. 2*D*) after administration of the nicotinic cholinoceptor antagonists, mecamylamine (2–10 mg/kg I.V.; 0·5–2 mg I.C.) and hexamethonium (5–15 mg/kg I.V.; 1–5 mg I.C.) or following local anaesthetization of the nerve trunk distal to the stimulating electrodes. Administration of atropine (1–2 mg/kg I.V.) did not affect type I responses.

Type I responses could still be obtained after administration of large doses of the α-adrenoceptor antagonists, phentolamine (5–15 mg/kg I.V.; 1–5 mg I.C.) and phenoxybenzamine (10–20 mg/kg I.V.): Considerably smaller doses (2–5 mg I.V.) abolished the dilatation of the pupil during stimulation of the preganglionic sympathetic trunk. The excitatory changes of chemosensory discharges to noradrenaline (1–5 μg I.C.) were also abolished or markedly attenuated by these smaller doses of α-blockers.

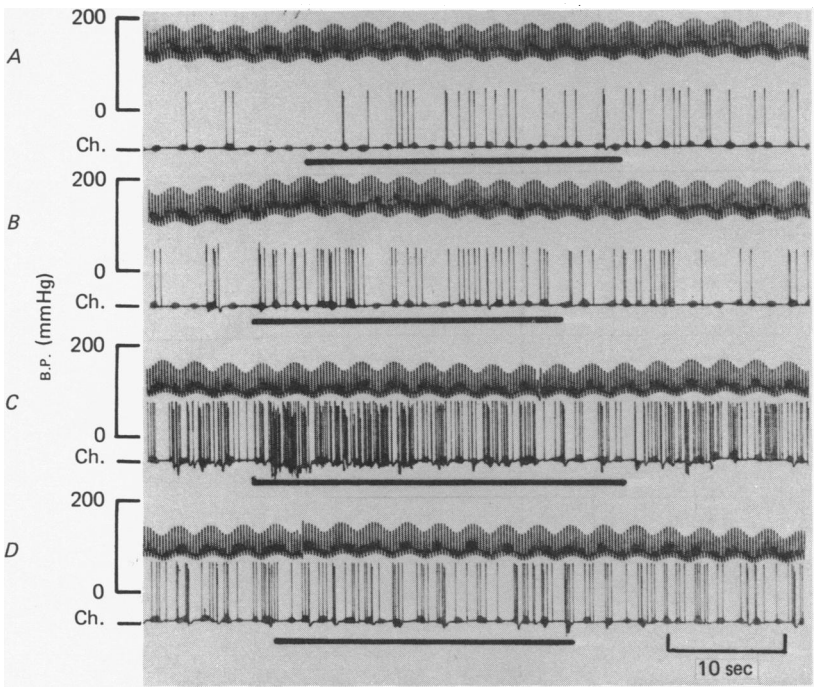

Fig. 2. Recordings of chemosensory activity (Ch.) and systemic arterial blood pressure (B.P.). Periods of electrical stimulation (9 V, 20 Hz, 1 msec) of the preganglionic sympathetic trunk are indicated by the bars underneath each tracing. *A*, control recording; *B*, recording taken 21 min after an I.C. injection of haloperidol (0·25 mg); *C*, recording taken 30 min after another I.C. injection of haloperidol (0·4 mg); *D*, recording taken 5 min after an I.C. injection of mecamylamine (1 mg).

Injections of α-adrenoceptor antagonists were followed, like those of haloperidol, by reductions in the levels of B.P. Chemosensory discharge frequencies also increased, sometimes to a considerable extent. In order to produce more comparable conditions for the study of the effects of drugs on chemosensory responses to sympathetic stimulation, the carotid body was artificially perfused in seven cats. In three cats it was possible by adjustment of the levels of perfusion pressure to produce approximately the same prestimulatory levels of chemosensory impulse frequency and a typical example of the results obtained from one of these experiments can be seen in Fig. 3. In the intervening periods between the six episodes of artificial perfusion, the carotid body was naturally perfused at the prevailing level of B.P. and drugs were administered during these periods. In the first period of artificial perfusion, before drug administration, preganglionic sympathetic stimulation caused a small excitation

of chemosensory discharges (Fig. 3*A*). This response was enhanced after administration of haloperidol (Fig. 3*B*) and was still elicited following two separate injections of phenoxybenzamine (Fig. 3*C*, *D*). Excitatory changes during stimulation resisted β-adrenoceptor antagonism induced by propranolol (Fig. 3*E*), but did not occur after administration of hexamethonium (Fig. 3*F*).

Type II excitatory responses. Type II excitatory responses were characterized by (*a*) accentuations of chemosensory impulse frequency which usually became more pronounced as stimulation progressed, and (*b*) susceptibility to α-adrenoceptor antagonism. These responses were either unaffected or slightly reduced in magnitude

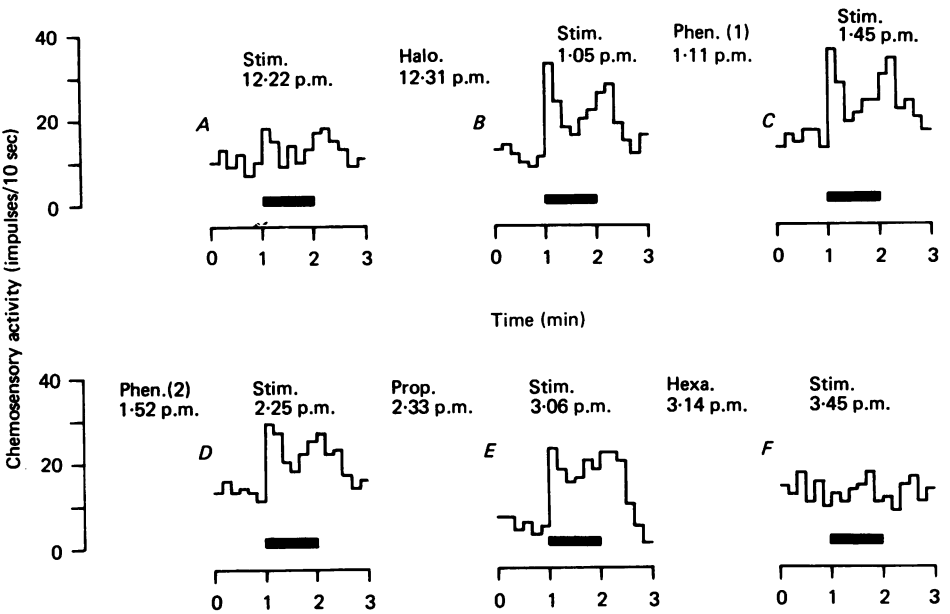

Fig. 3. Frequency of chemosensory impulses recorded from a filament of the cut sinus nerve plotted against time during periods of artificial perfusion of the carotid body (*A*–*F*). The periods of electrical stimulation (10 V, 20 Hz, 1 msec) of the preganglionic sympathetic trunk are indicated by the bars underneath the plots of impulse frequency. The times during the experiment when stimulation (Stim.) was initiated and drugs administered are indicated above and between plots. Halo., haloperidol (1 mg/kg I.V.); Phen (1), phenoxybenzamine (5 mg/kg I.V.); Phen. (2), phenoxybenzamine (10 mg/kg I.V.); Prop., propranolol (1 mg/kg I.V.); Hexa., hexamethonium (5 mg I.C.). (Consult text for further explanation.)

following haloperidol administration and were usually elicited when the mean levels of B.P. were less than 90 mmHg. Fig. 4*A* shows a typical example of a type II response obtained from an animal with systemic hypotension. The increase in chemosensory discharge rates began within 10 sec of the onset of stimulation and became more marked as stimulation progressed. Impulse frequencies gradually recovered to prestimulatory values within 60 sec of the end of stimulation. When the period of stimulation was more prolonged than that shown in Fig. 4*A*, it was found that discharge rates eventually reached a plateau of increased value within 45–75 sec of the onset of stimulation. This type of excitatory response was not affected to any significant degree by haloperidol administration (Fig. 4*B*) but was susceptible

to α-adrenoceptor antagonism (Fig. 4*C*), induced by either phentolamine (2– 5 mg/kg I.V.; 0·5–1 mg I.C.) or phenoxybenzamine (3–5 mg/kg I.V.).

Type II responses were unaffected by atropine administration (1–2 mg/kg I.V.), slightly reduced in magnitude after I.V. injections of propranolol (1 mg/kg) and no longer elicited following administration of nicotinic cholinoceptor antagonists in similar doses to those stated previously. Type II excitatory changes, like type I responses, were abolished by local anaesthetization of the preganglionic trunk distal to the stimulating electrodes.

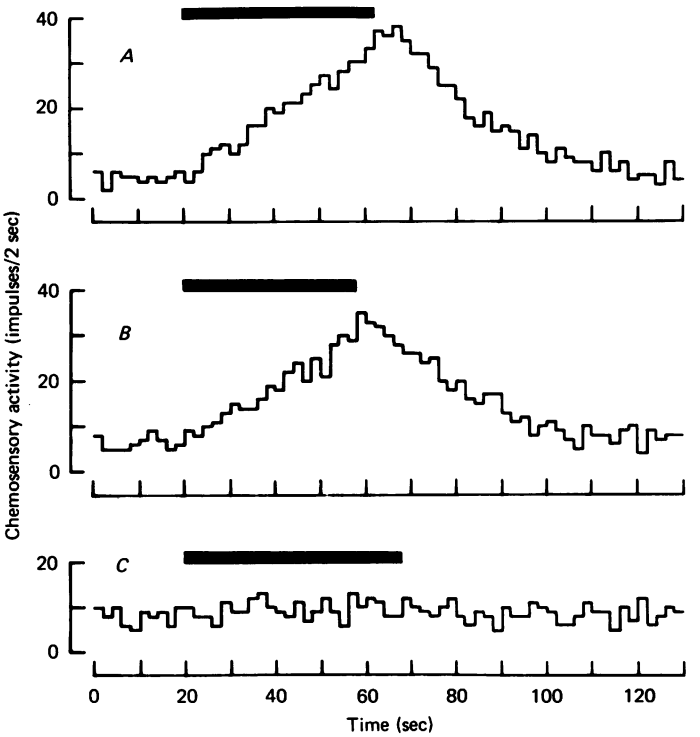

Fig. 4. Frequency of chemosensory impulses recorded from a filament of the cut sinus nerve plotted against time. Periods of electrical stimulation (8 V, 20 Hz, 1 msec) of the preganglionic sympathetic trunk are indicated by the bars. *A*, control recording; *B*, recording taken 20 min after an I.C. injection of haloperidol (0·5 mg); *C*, recording taken 15 min after an I.C. injection of phentolamine (0·5 mg).

Inhibitory responses

The discharges of thirteen chemosensory preparations were suppressed during stimulation of the preganglionic sympathetic trunk. This effect was no longer elicited after local anaesthetization of the preganglionic nerve trunk distal to the stimulating electrodes but reappeared when the local anaesthetic was washed away from the nerve trunk by saline lavage. This measure did not abolish inhibitory changes during preganglionic sympathetic stimulation in a further twelve preparations and these changes probably arose from current spread to either the carotid body or the chemosensory filaments (Eyzaguirre & Koyano, 1965; Fidone & Sato, 1970). These changes were also resistant to nicotinic cholinoceptor antagonism.

An example of the usual pattern of inhibitory responses to sympathetic stimulation (six preparations) can be seen in Fig. 5. Discharge rates were markedly reduced relative to control values in the initial stages of stimulation (Fig. 5*A*). As stimulation progressed, reductive changes became less pronounced. Recovery to the control values occurred within 5 sec of the end of stimulation (Fig. 5*D*). In four preparations depressive effects were limited to the initial 5–15 sec of sympathetic stimulation (see Fig. 7*A*). Three preparations showed the same degree of inhibition throughout stimulation.

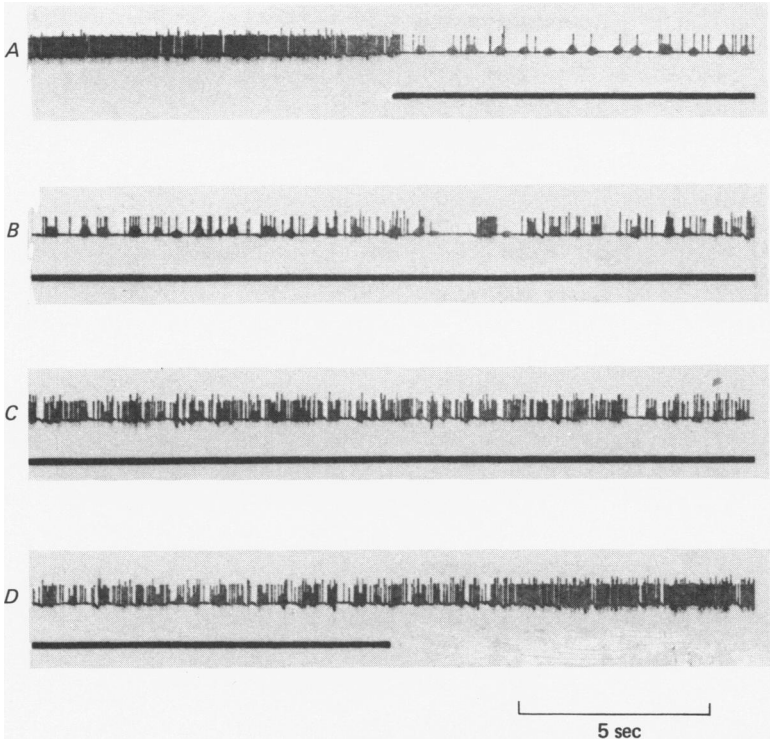

Fig. 5. Chemosensory activity recorded from a filament of the cut sinus nerve. The period of electrical stimulation (8 V, 20 Hz, 1 msec) of the preganglionic sympathetic trunk is indicated by the bar underneath the recording. *A* to *C*, continuous recording.

In ten chemosensory preparations administration of haloperidol (1–2 mg/kg I.V.; 0·3–0·6 mg I.C.) abolished inhibitory responses to sympathetic stimulation. In seven of these, type I excitatory responses replaced the inhibitory changes (see Fig. 7). Larger doses of haloperidol (1–2 mg I.C.) did not affect inhibitory responses in three preparations but these responses were no longer elicited after administration of mecamylamine (1 mg I.C.). Inhibitory responses resisted atropine administration (1 mg/kg I.V.) and adrenoceptor antagonism (α, β).

2. *Effects of electrical stimulation of the distal end of the cut ganglioglomerular nerve on chemosensory discharges*

The results of ganglioglomerular nerve stimulation obtained in this and another study are presented in Table 1. Overall, a statistical analysis showed that of the

thirty-nine preparations examined, eighteen underwent excitatory changes while three exhibited depressive effects.

In this investigation, excitatory responses to ganglioglomerular nerve stimulation were divided into types I (ten preparations) and II (three preparations) based on the criteria previously used. Type I responses obtained from three preparations were greatly attenuated after administration of mecamylamine (0·5–1 mg I.C.). Hexamethonium (5–10 mg I.C.) and higher doses of mecamylamine (3–5 mg I.C.) did not affect type I responses recorded from a further four preparations. Administration of these agents greatly reduced or abolished the excitatory effects of acetylcholine (5–20 μg I.C.) on chemosensory discharges.

The closeness of the stimulating electrodes on the ganglioglomerular nerve to the carotid body and the chemosensory filaments predisposed the preparations to suppression of discharges as a result of current spread. Such effects, observed in ten preparations, were no longer elicited after the stimulating electrodes were repositioned on the nerve. Inhibitory changes during stimulation obtained from three preparations were abolished by haloperidol administration and such changes cannot be regarded as due to current spread.

3. *Effects of electrical stimulation of the distal end of the cut preganglionic cervical sympathetic trunk on carotid body blood flow*

In seventeen cats stimulation of the preganglionic sympathetic trunk reduced carotid body blood flow, and this effect was observed during both natural and artificial perfusion of the carotid body (Fig. 6). In three cats a short-lasting vasodilator effect of small magnitude in the initial stages of stimulation preceded the reductive changes. In fourteen cats, with the mean levels of B.P. between 100 and 130 mmHg, the control values of carotid body blood flow ranged between 32 and 55 μl./min, with a mean value of 41 μl./min ($\pm$6·5, S.D.). These values are reasonably in line with those reported by Purves (1970*a*) in a larger series of animals. Carotid body blood flow was reduced from control levels by between 9 and 25 μl./min during stimulation. The mean change of 14 μl./min ($\pm$5·2, S.D.) represents a reduction of 34% from control values although changes as low as 20% and as high as 70% were noted. The magnitude of the reductive effect of sympathetic stimulation on blood flow has been underestimated to some extent because a new steady state was not attained during the course of stimulation in six cats (Fig. 6). In two cats control levels of blood flow were higher (60 and 85 μl./min respectively) as were the mean levels of B.P. (> 140 mmHg). Sympathetic stimulation caused blood flow to be reduced to between 40 and 50% of the control values. In another cat, with a mean value of B.P. of 80 mmHg, the low control level of carotid body blood flow of 17 μl./min decreased to 10 μl./min during sympathetic stimulation.

Effects of α-adrenoceptor antagonists. In ten cats the reductive effects of sympathetic stimulation on carotid body blood flow were either considerably attenuated or abolished after administration of either phenoxybenzamine (3–5 mg/kg I.V.) or phentolamine (2–5 mg/kg I.V.; 0·3–1 mg I.C.). Reductions, often marked, in the levels of B.P. and carotid body blood flow occurred after injections of α-blockers. Artificial perfusion of the carotid body was carried out in seven cats so as to produce more comparable conditions for the study of sympathetic effects before and after

injections of these agents. In three cats stable values of blood flow were achieved during periods of artificial perfusion. In the example shown in Fig. 6*A*, sympathetic stimulation decreased carotid body blood flow during both natural (●) and artificial (○) perfusion before administration of phenoxybenzamine. After administration of the drug, blood flow fell from 44 to 23 μl./min in association with a reduction of the mean levels of B.P. from 120 to 90 mmHg. During natural perfusion a small reduction of carotid body blood flow occurred during sympathetic stimulation (Fig. 6*B*, ●). The level of perfusion pressure was adjusted during artificial perfusion to give approximately the same values of blood flow as those which pertained before injection of phenoxybenzamine. Under these circumstances, sympathetic stimulation caused a small reduction of carotid body blood flow (Fig. 6*B*, ○) but this effect was much less than before administration of the drug (Fig. 6*A*, ○).

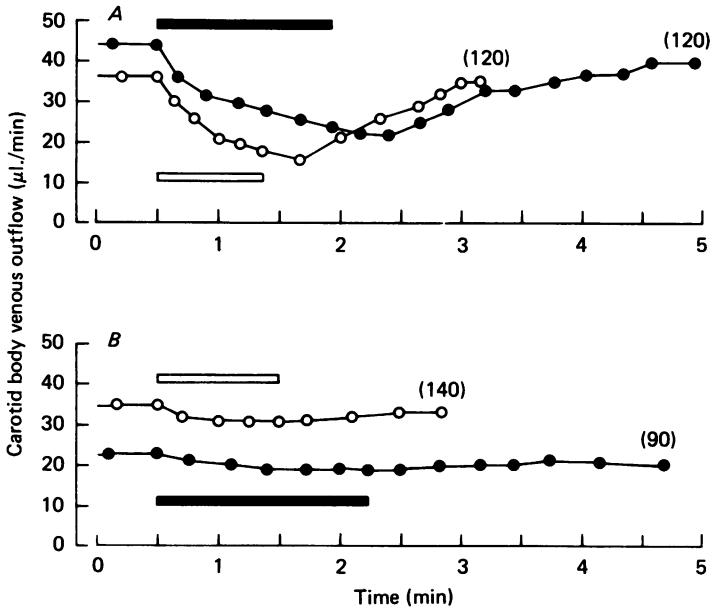

Fig. 6. Measurements of the rates of carotid body blood flow during periods of natural (●) and artificial (○) perfusions of the carotid body. Periods of electrical stimulation (9 V, 20 Hz, 1 msec) of the preganglionic sympathetic trunk are indicated by the bars (filled bar, stimulation during natural perfusion; open bar, stimulation during artificial perfusion). Symbols in this and the subsequent Figure represent the flow rate (expressed in μl./min) for the preceding time taken to measure 5 or 2 μl. (depending on the rate of flow). The numbers in brackets refer to the levels of mean perfusion pressure measured by means of a lingual artery cannula. Between *A* and *B*, phenoxybenzamine (5 mg/kg) was injected I.V. (Consult text for further explanation.)

Effects of haloperidol and atropine. In five cats haloperidol injections (1–2 mg/kg I.V.; 0·1–0·5 mg I.C.) had no influence on or slightly decreased the reductive effects of sympathetic stimulation on carotid body blood flow (Fig. 7). Vasodilator effects to sympathetic stimulation were unaffected by administration of atropine (1–2 mg/kg I.V.).

Measurements of carotid body blood flow and chemosensory discharges in the same experiment. In six cats blood flow through the left carotid body was measured simultaneously with chemosensory discharges recorded from filaments prepared from the ipsilateral sinus nerve. In all experiments stimulation of the preganglionic sympathetic trunk reduced carotid body blood flow and increased chemosensory discharges. Administration of α-adrenoceptor antagonists abolished or greatly decreased the changes in blood flow but did not affect the excitatory effects on discharges. An example from one of these experiments can be seen in Fig. 7. Before injection of drugs, sympathetic stimulation reduced carotid body blood flow but had little effect on

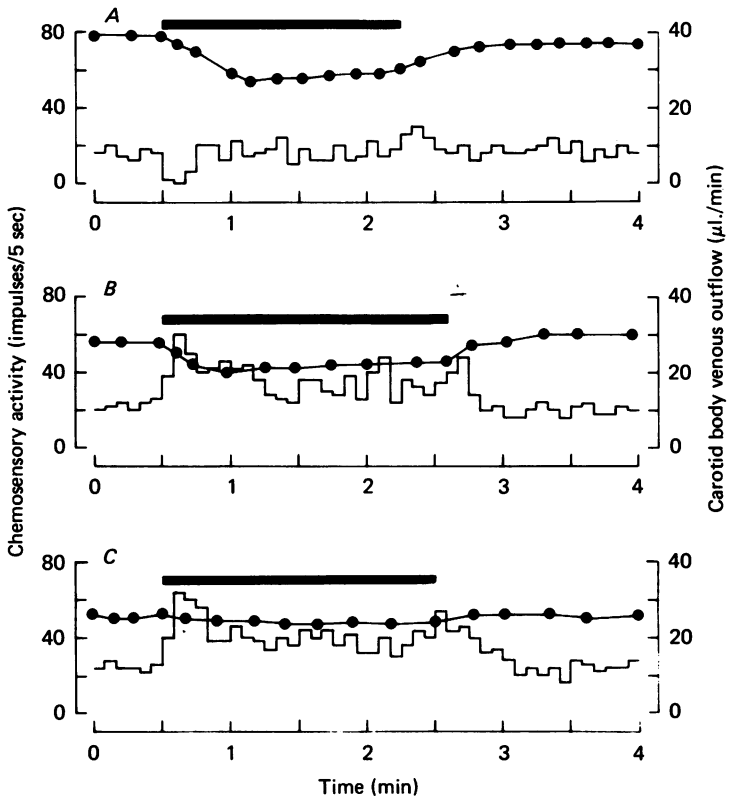

Fig. 7. Carotid body blood flow (●) and chemosensory impulse frequency (histogram arrangement) measured simultaneously. Periods of electrical stimulation of the preganglionic sympathetic trunk are indicated by the bars. *A*, control measurements; *B*, measurements obtained 20 min after an I.C. injection of haloperidol (0·5 mg); *C*, measurements obtained 10 min after an I.C. injection of phentolamine (1 mg).

chemosensory discharges apart from a transient inhibition limited to the initial stages of stimulation (Fig. 7*A*). After an I.C. injection of haloperidol the reductive effects of sympathetic stimulation on blood flow were slightly reduced (Fig. 7*B*) and the inhibitory effects upon chemosensory discharges were replaced by type I excitatory responses. Following administration of phentolamine chemosensory excitation still occurred during stimulation although the reductive changes in blood flow were virtually abolished (Fig. 7*C*).

DISCUSSION

This investigation indicates that activation of the sympathetic supply to the carotid body can influence chemoreceptor activity in a more complex manner than hitherto realized. Two categories of excitatory responses to sympathetic stimulation, for convenience designated as types I and II, were distinguished. Occasionally, sympathetic stimulation caused a suppression of chemosensory discharges. Chemosensory excitation mediated by sympathetic fibres has been generally considered to be the consequence of vasoconstriction. However, type I excitatory responses to sympathetic stimulation, as observed in this investigation, are highly unlikely to have been due to blood flow changes. The temporal pattern of responses of carotid body blood flow and type I excitatory effects to sympathetic stimulation bore little relationship to one another when these responses were measured together in the same experiment. Furthermore, type I responses were unaffected by administration of doses of α-adrenoceptor antagonists well in excess of those needed to abolish or considerably reduce the vasoconstrictor effects to sympathetic activation. Additionally, haloperidol injections enhanced type I responses or caused their appearance for the first time but had little influence on blood flow changes during sympathetic stimulation. The existence of non-vascular mechanisms of modulation by sympathetic nerves is also indicated by the findings of another investigation (Acker & O'Regan, 1981). It was found that sympathetic stimulation caused reductions, often marked, in the rates of venous outflow from the carotid body, yet had little effect on tissue P_{O_2} or local flow monitored by electrodes inserted into the organ. Moreover, sympathetic stimulation could excite chemosensory discharges in the presence of an unchanged tissue P_{O_2}. It would appear that the major control by sympathetic nerves is exerted on shunt vessels.

Non-vascular sympathetic effects imply the presence of sympathetic fibres innervating glomeral structures apart from blood vessels. A number of histological studies have demonstrated in a variety of animals the existence of noradrenergic sympathetic nerve endings in close proximity to both types of glomeral cells and also to sensory nerve terminals (Korkala, Eränkö, Partanen, Eränkö & Hervonen, 1973; Verna, 1975; Kobayashi, 1976; Knoche & Kienecker, 1977; Vásquez-Nin, Costero, Echeverria, Aguilar & Barroso-Moguel, 1978). Because these post-ganglionic fibres are noradrenergic, their activation during sympathetic stimulation could not have been responsible for type I excitatory responses, as these responses resisted α-adrenoceptor antagonism. However, they could have been implicated in the generation of type II excitatory responses (see later). A nerve supply to the type I cells of the rat consisting of preganglionic cervical sympathetic fibres has been described by McDonald & Mitchell (1975) and by Kondo (1976). While McDonald & Mitchell postulate that the function of this innervation is to cause release of dopamine from type I cells which then suppresses the activity of the chemosensory terminals, no evidence has been presented to support this view. Such a nerve supply could be considered as a possible candidate for explaining type I excitatory responses. These sympathetic fibres course to the carotid body in the ganglioglomerular nerve so electrical stimulation of this nerve should activate them, and being preganglionic fibres their influences within the carotid body should be affected by nicotinic cholinoceptor antagonists. Administration of these antagonists left most of the type I responses to ganglioglomerular nerve

stimulation unaffected although the enhancing effects of I.C. injections of acetylcholine on chemosensory discharges were either abolished or considerably reduced in magnitude. Sympathetic ganglion cells have been described as being present both in the ganglioglomerular nerve and in the carotid body (deCastro, 1926; McDonald & Mitchell, 1975) and nicotinic cholinoceptor antagonists could have affected transmission between preganglionic fibres and these cells. Such an action could explain the attenuation of some type I responses to ganglioglomerular nerve stimulation. Therefore, evidence would appear to be against a role for preganglionic sympathetic fibres in the generation of type I responses. Furthermore, in the cat the ganglioglomerular nerve contains few myelinated axons (Eyzaguirre & Uchizono, 1961) and section of the preganglionic cervical sympathetic trunk has not been shown to lead to degeneration of nerve terminals on the type I cells (Biscoe & Stehbens, 1967).

Recent investigations (Lundberg, Hökfelt, Fahrenkrug, Nilsson & Terenius, 1979; Wharton, Polak, Pearse, McGregor, Bryant, Bloom, Emson, Bisgard & Will, 1980) have shown an extensive network of nerve fibres exhibiting immunoreactivity to vasoactive intestinal peptide (VIP) and substance P in the carotid body. These fibres were found in association with blood vessels and type I cells. While the origin, nature and ultimate destination of these fibres has yet to be elucidated they could be regarded as possible mediators of type I excitatory responses to sympathetic stimulation, especially as both VIP and substance P have been shown to excite chemosensory discharges (Said & Mutt, 1970; McQueen, 1980).

It is difficult to form an opinion as to how sympathetic fibres with non-vascular terminations modulate chemoreceptor activity, owing to the considerable confusion which currently exists concerning the mechanism of chemoreception within the carotid body. Type I cells, type II cells and sinus nerve chemosensory terminals have all been postulated as comprising the primary chemosensitive elements (for recent review see Verna, 1979) and sympathetic influences could be exerted on any of these elements to modulate their activity.

In contrast to type I responses, type II excitatory changes could have been the consequence of associated blood flow changes during sympathetic stimulation. In a number of experiments sympathetic stimulation caused changes in carotid body blood flow whose temporal pattern of response was like that which would be expected if vasoconstrictor effects were responsible for type II excitatory responses. Administration of approximately the same doses of α-adrenoceptor antagonists abolished or greatly decreased both type II responses and the reductive changes of carotid body blood flow during sympathetic stimulation. Type II responses to sympathetic stimulation were usually associated with low levels of B.P. Because noradrenergic fibres have been found close to and even in synaptic contact with type I cells, it cannot be excluded that activation of these fibres during sympathetic stimulation caused at least some of the α-adrenergic excitatory effects observed in the present investigation. In favour of such a possibility is the finding that local flow and tissue P_{O_2} within the carotid body are little affected by sympathetic stimulation even in hypotensive circumstances (Acker & O'Regan, 1981).

The conclusion that most of the excitatory responses to sympathetic stimulation are not due to changes in carotid body blood flow is at variance with the opinion expressed in other reports. Eyzaguirre & Lewin (1961) and Belmonte & Eyzaguirre (1974) concluded that vascular changes could explain all excitatory effects mediated

by sympathetic fibres on the basis that sympathetic stimulation did not influence discharges in the *in vitro* superfused carotid body preparation. However, it is doubtful whether the *in vitro* preparation is suitable for the study of responses dependent on catecholaminergic mechanisms. As an example, it was found that dopamine was either ineffective (Zapata, Hess, Bliss & Eyzaguirre, 1969) or depressed chemosensory discharges only on the first application (Zapata, 1975) when the *in vitro* preparation was used. Furthermore, applications of other catecholamines to the *in vitro* preparation altered the responses to dopamine. Yet, it is well known that injections (I.C., I.V.) of dopamine can repeatedly inhibit discharges recorded from carotid bodies perfused with blood (Black, Comroe & Jacobs, 1972; Sampson, 1972; Llados & Zapata, 1978), an effect which is independent of blood flow changes (Sampson, Aminoff, Jaffe & Vidruk, 1976). The findings obtained from *in vivo* blood-perfused organs would seem to be the more reliable and, indeed, the effects of catecholamines obtained from such preparations are now universally accepted. Another drawback of the *in vitro* preparations is that in order to produce optimal conditions for oxygenation it is necessary to remove extensively the connective tissue capsule of the organ. Sympathetic fibres destined to supply the carotid body are present in a network in this connective tissue capsule (deCastro, 1926) and could be destroyed during preparations. Attempts were made to repeat the findings of McCloskey (1975) that the rates of development of stagnant asphyxial chemosensory discharges to an abrupt abolition of carotid body blood flow were unaffected by sympathetic stimulation. However, it was found that the patterns of development of discharges varied widely with individual trials (unpublished observations), so conclusions based on such experiments are open to question. Furthermore, there is difficulty in distinguishing excitatory effects during sympathetic stimulation when discharge rates are high (Belmonte & Eyzaguirre, 1974), as during stagnant asphyxia.

Electrical stimulation of the preganglionic sympathetic trunk caused reductions of impulse frequencies recorded from 20% of chemosensory preparations. However, some of these inhibitory responses to sympathetic stimulation probably arose from current spread during stimulation either to the carotid body or the chemosensory filaments. Inhibition of discharges due to activation of sympathetic fibres during stimulation was recorded in 10% of preparations. However, further evidence for the existence of such fibres is indicated by the results of haloperidol administration. Following administration of this agent, sympathetic stimulation caused type I excitatory responses in chemosensory preparations which had previously been unaffected during stimulation. It would seem that electrical stimulation of sympathetic nerves supplying the carotid body can activate different groups of nerve fibres some of which cause excitation, others inhibition, and depending on the balance of fibres activated, chemoreceptor activity could be enhanced or reduced or show little change. Haloperidol, by abolishing inhibitory influences, permitted excitatory effects to manifest themselves fully during sympathetic stimulation.

The effects of haloperidol indicate a role for dopamine in the generation of inhibitory responses. Dopamine is mainly located in type I cells but its function in chemoreception is obscure (see Mills, Smith, Slotkin & Breese, 1978). However, it is likely, as postulated by McDonald & Mitchell (1975), that sympathetic stimulation induced a release of dopamine from the type I cells which then suppressed the activity

of the chemosensory terminals. The involvement of a preganglionic cervical sympathetic supply to the type I cells is improbable because, as previously discussed, evidence for such a nerve supply in the cat is lacking at present.

This investigation confirmed the vasoconstrictor effects of sympathetic stimulation on carotid body blood flow (Daly *et al.* 1954; Purves, 1970*b*) and showed that this effect was susceptible to α-adrenoceptor antagonism. Small vasodilator effects limited to the initial stages of sympathetic stimulation were also noted and this effect could be explained by the existence of post-ganglionic cholinergic fibres supplying carotid body blood vessels, as has been suggested by Biscoe & Silver (1966) from their studies on the distribution of cholinesterase in the carotid body. However, atropine did not affect the vasodilator effects to sympathetic stimulation so muscarinic cholinoceptor mechanisms were not involved.

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